IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Peter ERIKSSON

Application No.: 09/856,576

Filed: July 13, 2001

For: MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS

Group Art Unit: 1647

Examiner: Christopher J. Nichols, Ph.D.

Confirmation No.: 9724

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter Eriksson declare as follows:

(1) I reside at Gothenburg.

(2) I am a citizen of Sweden.

(3) I have been employed at Göteborg University since 2001 as a Professor in the Department of Clinical Neuroscience.

(4) I am the named inventor on the above-referenced application.

(5) I am an expert in the field of neuronal stem cell biology as substantiated by my curriculum vitae (attached hereto).

(6) I am familiar with the above-referenced patent application and the Office Actions dated November 21, 2003 and June 10, 2003 (Paper No. 10). Based on the Office Action, it is my understanding that the Examiner alleges that the claims of the present invention lack a disclosure that is adequate to enable a skilled person to make or use the invention.

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(7) I note that the claims of the present invention are directed to methods of propagating cells selected from the group consisting of neuronal progenitor cells and neuronal stem cells by administration of a composition comprising an amount of a growth hormone effective to propagate neuronal progenitor cells and neuronal stem cells *in vitro*. The invention also relates to a method of propagating cells selected from the group consisting of neuronal progenitor cells and neuronal stem cells in, or derived from, the central or peripheral nervous system in a patient in need of neuron propagation.

REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

(8) Claims 53 and 59 were rejected for lack of enablement, because the Examiner believed that the data presented in the specification was insufficient. Specifically, the Examiner questioned whether growth hormone treatment actually induced lineage determination or neurogenesis.

(9) Claims 53 and 59 have been amended to recite that the cells are propagated, in view of the *in vivo* data provided in this patent application as set forth on pages 10 to 14. In my opinion the data provided in this example supports the invention, i.e. that administration of growth hormone induces proliferation of neuronal progenitor cells and neuronal stem cells from the central nervous system (exemplified by the dentate gyrus). Accordingly, based on these data, I believe that a skilled artisan will understand that it is possible to propagate neuronal progenitor cells and neuronal stem cells by the use of growth hormone.

(10) Additionally, I disagree with the Examiner that the example provided in the application is a non-specific test of cell proliferation. The Examiner asserted that it is non-specific, because BrdU incorporation is a non-specific test and thus the observed result could be due to cell proliferation of glia and not due to proliferation of neurons *per se*. The following data demonstrates that growth hormone (GH) treatment results in an increase in neuronal progenitor cell and neuronal stem cell proliferation and not an increase in glia cell proliferation.

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(11) The experiment to test the proliferation of neurons was performed as follows. We investigated the effects of peripheral administration of bovine GH (bGH) on the proliferation of stem/progenitor cells in the adult rat hippocampus. Hypophysectomized (hx) female Sprague Dawley rats were maintained with cortisol (400 microg/kg x day) and L-thyroxine 4 (L-T4) (10 microg/kg x day). These animals were then treated with bGH (1 mg/kg x day) for 19 days. All treatments were given via subcutaneous injections. Tissue sections were treated for DNA denaturation, as described in the example on pages 10 to 14 of the above-identified application. The samples were then incubated in 0.25% Triton X-100 and 3% normal horse serum (TBS-TS) for 30 minutes. Thereafter, the samples were incubated overnight at 4°C with rabbit anti-Calbindin D_{28K} (1:500 dilution; SWant, Bellinzona, Switzerland), mouse neuronal nuclear protein (NeuN) (1:30 dilution; Chemicon, Temecula, CA), rabbit anti-Glial acidic fibrillary protein (GFAP) (1:500 dilution; Dako, Glostrup, Denmark), or rat anti-Bromodeoxyuridine (BrdU) antibody (1:200 dilution; Harlan, Loughborough, United Kingdom). GFAP and Calbindin D_{28K} were detected with Texas Red-conjugated anti-rabbit immunoglobulin (IgG) (1:200 dilution for GFAP and 1:100 dilution for Calbindin D_{28K}; Jackson ImmunoResearch, West Grove, PA). Calbindin D_{28K} was detected with Cy5-conjugated anti-rabbit IgG (1:150 dilution; Jackson ImmunoResearch). NeuN was detected with Texas Red-conjugated anti-mouse IgG (1:100 dilution; Jackson ImmunoResearch). BrdU was labeled with an fluoresceinisothiocyanate (FITC)-conjugated anti-rat IgG (1:150 dilution; Jackson ImmunoResearch) for 2 hours at 37°C. Immunofluorescence was detected with a Nikon Diaphot fluorescence microscope and confocal laser scanning microscopy using a BioRad 1024 system (Hercules, CA) according to manufacturer's instructions.

(12) The co-localization of BrdU was determined with cell-specific markers in the GCL (ganglion cell layer) in six to eight 40 μm thick coronal sections taken 240 μm apart in each animal. For the neuronal marker Calbindin D_{28K}, 511 BrdU-positive cells were analyzed with respect to co-localization. For the neuronal marker NeuN, 748 BrdU-positive cells were analyzed. For the glial marker GFAP, 902 BrdU-

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positive cells were analyzed. All cell-counting procedures were blindly performed. Co-localization of BrdU immunoreactivity with immunoreactivity of the granule cell marker Calbindin D_{28K} and the astrocyte marker GFAP was investigated to determine the phenotype of progenitor cell progeny in the dentate gyrus after long-term growth hormone (GH) therapy compared with that of hx control animals. Using confocal microscopy, we were able to detect co-localization of BrdU with either Calbindin D_{28K} or GFAP in the GCL.

(13) In GH treated hx animals, the Calbindin D_{28K}- and BrdU-immunoreactive cell fraction was observed to be 41%±4% in GH treated and 43%±5% (NS) in hx control animals. In addition, 17%±3% of BrdU-positive cells were also GFAP positive compared with 19%±4% in hx control animalss (NS). To confirm that the increase of BrdU- and Calbindin D_{28K}-immunolabeled cells represented new neurons, additional double-immunolabeling characterizations with the neuronal marker NeuN were performed. Equal fractions of BrdU- and NeuN-labeled cells (*i.e.*, 54%±5.0% and 55%±4.0%, respectively) were observed in GH-treated animals as compared with untreated controls. The fraction of neurons and glial cells were thus unaltered by the treatment with growth hormone.

CONCLUSIONS

(14) Thus, considering that the number of newborn stem cell progeny was increased after GH treatment as discussed in the example on pages 10-14 of the above-identified application, and that the resulting fraction of neurons and glial cells were unaffected (as demonstrated in data described above), it can be concluded that GH treatment increases neurogenesis through an increase in neuronal progenitor cell and neuronal stem cell proliferation and not due to an increase in glial cell proliferation.

(15) I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful

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false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

2004-04-19
Date

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Ulrika Karlsson
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Curriculum Vitae

Peter S. Eriksson

Address: Institute of Clinical Neuroscience, Department of Experimental Neuroscience, Sahlgrenska University Hospital, 413 45 Göteborg, Sweden.

D.O.B: June 5th 1959, Göteborg, Sweden

Summary of research:

Principal investigator of the Neural Stem Cell laboratory, Sahlgrenska University Hospital, founded 1996, currently involving 19 co-workers with main focus on basic mechanisms for stem cell based therapy and repair in the central nervous system. The laboratory has an internationally leading position. First group ever to demonstrate that the human brain contain stem cells generating neurons throughout life (Eriksson et al., Nature med. 1998). Multiple research awards.

Education:

Docent in Neurobiology at the Medical faculty, Göteborg University, 1999.

Doctor of Medicine (Ph.D.) Göteborg University. External examiner: Dr. Michael D. Norenberg, University of Miami, U.S.A., 1992

University medical degree (MD) Faculty of Medicine, Göteborg University, 1987.

Naval Officer, Royal School of Naval warfare, Stockholm, 1981.

Academic and Clinical Positions:

2000-	Professor and Senior specialist in Neurology at the Institute of Clinical Neuroscience, Sahlgrenska University hospital.
1998-.	Combined position: Assistant professor/residency in neurology at the Institute of Clinical Neuroscience, section for experimental neuroscience, Sahlgrenska University Hospital, Sweden.
1997	One year's full research grant from Hjärnfonden, Inst. of Clinical Neuroscience, Dept. of Neurology, Göteborg University, Sweden.
1995	Postdoctoral position with Prof. Fred Gage, Salk Inst., San Diego, California.
1994 -	Residency at the Department of Neurology, Sahlgrenska University Hospital, Göteborg Sweden.
1992-1994	Clinical rotation program, Sahlgrenska University Hospital, Göteborg Sweden.
1987-1992	Graduate student position, full time. Dept. of Histology, Inst. of Neurobiology, Göteborg University.
1987	Physician at Dept. of Psychiatry at Lillhagens Sjukhus.
1982-1987	Graduate student position, part time. Dept. of Histology, Göteborg University.

Post Doctoral supervision:

Michelle Anderson, Adelaide Australia.
Ann-Catrin Thoresson, Sweden.
Thorleif Thorlin, Sweden.
Rogan Tinsley, Australia.
Maria Åberg, Sweden.
David Åberg, Sweden.
Anders Persson, Sweden.

Ph.D. students:

Supervisor for Thorleif Thorlin, Graduate studies doctoral dissertation 23-10-98
Supervisor for David Åberg, Graduate studies, doctoral dissertation 19-04-01.
Supervisor for Maria Åberg, Graduate studies, doctoral dissertation 20-04-01.
Supervisor for Anders Persson, Graduate studies, doctoral dissertation during 28-11-03.
Supervisor for Ekaterina Perfilieva, Graduate studies. licentiate dissertation during 2004.
Supervisor for Annica Dahl, Graduate studies.
Supervisor for Mila Komitova. Graduate studies, planned doctoral dissertation during fall 2004.
Supervisor for Jenny Nyberg, Graduate studies, Licenciate dissertation 23-10-03.
Supervisor for Matilda Zetterström, Graduate studies.
Supervisor for Jonas Faijersson, Graduate studies.
Supervisor for Ulf Johansson. Graduate studies, 10 University points, August 2000, "IGF-I induces proliferation in the rat subventricular zone.
Supervisor for Nina Hellström 20 University points February 2001, Selective expression of GH in the mouse brain induces neurogenesis in the adult dentate gyrus.
Supervisor for Jonas Faijersson 20 University points February 2002, Expression of neuronal markers in mesenchymal stem cells.

Teaching; formal training:
Extensive theoretical and practical education in pedagogy at the Royal School of Naval Warfare (1980-1981).
Administration of academic courses:

1999 - Member of the coordinating committee at NCG (Neurocentrum Göteborg) for neuroscience research courses at Göteborg University.
1992 – 1994 Responsible for the course "Special Cell Biology" within the biomedical cell biology program at Sahlgrenska University Hospital.
1992 Planned and organized the new course "Special cell biology" within the 40 university point program "Biomedical cell Biology". This course included a number of novel pedagogic features, such as a dissertation-like examination procedure with opponents and respondents; problem based learning tutorials and individually assigned laboratory tasks.
1988 – 1991 Responsible for the course in human histology for both medical and dentistry students. Responsible for the educational material supplied to the medical students attending this course.

Academic teaching:
1992 - Lecturer in Human Histology, Histology of the central nervous system for medical students and students at the School of Dentistry at Göteborg University, Biomedical cell biology at the Biomedical research school (forskarskolan) and at the Pharmaceutical industry program (Läkemedelsprogramet).
1983 – 1991 Tutor and lecturer in Human Histology and in Histology of the central nervous system (every spring and fall semester) for medical students and for students at the School of Dentistry, Göteborg University.
Academic teaching - research courses:
1998 Lecture on "Molecular biology as tools in Neuroscience research" in Methods in Neurobiology (Forskarutbildningskurs i Neurobiologiska metoder), at the Faculty of Medicine, Inst. of Neurobiology, Göteborg University.
1994 Lecture on "The role of astroglial opioid receptors in excitatory control" in the research course: "Excitability, Excitotoxicity and Neurotoxicology" - Theory and Clinical practice, at the Dept. of Clinical Neuroscience, Section of Neurology at Sahlgrenska University Hospital, Göteborg.
Enrepreneurial achievements:
Co-Founder of and Member of the Board of Directors at Arvid Carlsson Institute. Co-Founder of and Member of the Board of Cell Therapeutics Scandinavia AB and Co-Founder of Sahltech AB. Member of the Board of Cellectricon AB.
Invited Lectures:
Stem cells in the adult brain, Plenary lecture at the 32nd meeting of the International Narcotics Research Conference, July 18, 2001 Helsinki.
Processer i Hjärnan, Från stamcell till symptom; Behandling idag och i framtiden.Janssen-Cilag. Sept. 27, 2001 Stockholm
Sven Järring symposium, European Academy of Childhood disability. From Stem Cells to Rehabilitation Oct 11-14. 2001 Göteborg.
Stem Cells in the mammalian brain – The 4th brain research interactive symposium, Nov. 8-10 2001, San Diego, CA USA.
Stem Cells - Tools and targets for brain repair. Future prospects of stem cells, August 28 2003 Kobe, Japan.
Stem Cells for brain repair. Genes and Behaviour, October 19, 2003 Almeria, Spain.

Honorary Lectures:
Invited to become " Honorary lecturer", at the University of Toronto, Canada, May 2000.
"Neurogenesis in the human CNS; future therapeutic implications" Collége de France, Paris, France, June 1999.
Physical Exercise and Neurogenesis in the Brain KI-PARC Annual Lecture October 2000.
Organization of Scientific Meetings:
Member of the Scientific organizing committee of the conference "Stem cell research from genes to patients Örenäs, September 2003.
Chairman of the organizing committee of the NIH symposium "Networking on human embryonic stem cell research Göteborg, September 2003.
Member of the organizing committee of the 1st Arvid Carlsson symposium October 2002.
Chairman of the organizing committee of the 5th Neuroscience day in Göteborg October 2000.
Chairman of the organizing committee of the 4th Neuroscience day in Göteborg October 1999.
Member of the organizing committee at the 1988 ESN meeting in Göteborg.

Scientific Awards:
Named first author of the most important scientific report in neuroscience during the decade of the brain (1990-1999) by the New York Times, January 2000.
Winner of the "Best of What's New" Award 1999, Popular Science Magazine.
Winner of the Folins prize for neurological research 1999.
Winner of the Jubilee prize of the Swedish Medical Society, October 2000.
Winner of the Swedish Parkinson Foundation honorary silver medal, April 2001.

Membership in scientific organizations:
Member of the board of Neurocentrum Göteborg (NCG).
Member of the Society for Neuroscience.
Member of Neurovetenskapliga föreningen, Göteborg.

National scientific collaborations:
Professor Keiko Funa at the department of Anatomy and Cell biology, University of Göteborg. Collaborative research on the role of Bone Morphogenic Factors (BMPs) in lineage determination in adult derived hippocampal stem cells.

Docent Björn Carlsson at the department of Internal Medicine, RCEM at Sahlgrenska University Hospital. Collaborative research on partial genome scale analyses of adult derived neuronal progenitors using Affymetrics technology.

Professor Barbro B. Johansson at Experimental Neurology, Wallenberg Neuroscience Center, University Hospital Lund. Collaborative research on the role of neural stem cells in experimental strokes in rats.

Professor Anders Lindahl at the Department of Clinical Chemistry, Sahlgrenska University Hospital. Collaborative research on the use of alternative tissue sources for stem cells.

Professor Torsten Ohlsson at the department of Applied Physics at Chalmers University of technology. Collaborative research on time lapse microscopy including advanced pattern recognition and tracking technology for the analysis of in vitro neural stem cells.

Docent Owe Orwar at the Department of analytical Chemistry at Göteborg University. Collaborative research on experimental methods for gene and nuclear transfer at the single cell level and research on methods to analyze the release of neurotransmitters from single cells.

Docent Jan Oscarsson at the department of Physiology and Pharmacology at the University of Göteborg. Collaborative research on the role of IGF-I in adult hippocampal neurogenesis and in Connexin-43 regulation.

Professor Lars Terenius and Co-workers at Center for Molecular Medicine, Karolinska Institute, Stockholm. Collaborative research on the role of opioid receptors on adult derived neural stem cells.

International Collaborations:
Professor Fred Gage at the Salk institute for biological studies, La Jolla, California, USA. Collaborative research on the regulation of neurogenesis in the primate (Vervet monkey) brain.

Professor A. John MacLennan at the department of Neuroscience, University of Florida, Brain Inst. College of Medicine, Gainesville, Florida, USA. Collaborative research on the signal transduction of Ciliary Neurotrophic Factor (CNTF) in adult derived hippocampal stem cells.

Professor Per Odin at Klinik Fur Neurologie der Philipps-Universität Marburg, Germany. Collaborative research on the use of adult derived stem/progenitor cells for the treatment of experimental Parkinsonian syndrome in rats.

Professor Theo Palmer at the department for Neurosurgery, Stanford University, California, USA. Collaborative research on IGF-I signal transduction in adult derived hippocampal Stem cells.

Professor A. Popa-Wagner at Klinik Fur Neurologie der Ernst-Arndt-Moritz-Universitaet, Greifswald, Germany. Collaborative research on the effects of enriched environment on stroke recovery in rats. One joint EU-grant request has been submitted as part of this collaboration.